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Dossier: Drug delivery and drug efficacy

Principles of transmucosal delivery of therapeutic agents

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Abstract

Recent advances in medicine have led to new treatment options for patients and physicians as a more developed understanding of the molecular basis of disease states is translated into new therapeutic agents. Many of these new agents are compounds that are not able to reach the bloodstream when administered by the oral route preventing the ability to enjoy the benefits this delivery route provides such as lower cost and increased quality of life. Our laboratory has focused on the use of hydrogel carriers to increase the bioavailability of orally administered therapeutic agents ranging from proteins such as insulin to chemotherapeutics like bleomycin. The foundations of this research as well as recent advances are reviewed along with a discussion of the challenges of oral administration and other emerging strategies for oral administration.

Keywords: Oral administration; Hydrogels; Insulin; Chemotherapy; Transmucosal delivery; Protein delivery

1. Introduction

Advances in medicine and science in the past century have caused a dramatic increase in the average life span of the human being. While medical science has found treatments for many diseases, a number of ailments remain where only alleviation of the symptoms can be achieved. With the development of protein synthesis technologies and the ability to mass-produce protein therapeutics, many individuals have access to treatments for once incurable diseases.

The emerging technique of combinatorial chemistry, along with a growing knowledge of the biochemistry of the human body, has lead to an ever-increasing number of therapeutic proteins in the treatment of diseases; however, these proteins often lack durability that more traditional small molecule pharmaceutics possess. Where a simple therapeutic agent, such as aspirin or simple antibiotic, can be taken orally and reach the bloodstream intact, the larger and more delicate protein must often be delivered directly into the bloodstream through injection. The harsh conditions of the stomach often destroy a vast majority of the protein before it reaches the bloodstream. In the case of insulin, less than 0.1% of the orally dosed insulin reaches the blood stream intact [1]. This means that for patients to make use of the ever-expanding protein database, they must administer the protein through injections.

Unfortunately, injections are often painful, which can lead to low patient compliance [2]. Thus, medical research has endeavored to find alternate ways of delivering proteins and other fragile therapeutics. One such route is oral delivery. Taking a pill for sickness or disease is not painful and much easier than dealing with injections. Thus, the patient compliance would increase, improving the efficacy of the treatment. Any company to develop such a system would almost certainly supplant the current injection-based industry. Thus, there is great interest in developing a method of oral protein delivery.

The benefits gained from a viable oral administration method also apply to other types of therapeutic agents as well as proteins. One example that will be discussed in detail is oral administration of chemotherapeutic agents. As discussed above, the environment of the gastrointestinal tract can be damaging to these agents but there are other concerns related to the toxicity that many cancer drugs exhibit towards healthy tissue. This adds an additional layer of complexity to the challenge of oral administration of these compounds.

Oral administration has numerous barriers to overcome in order to create an effective system for delivery of therapeutic agents. The greatest barriers are the harsh conditions of the

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stomach and the transport barrier, present in the intestines. The stomach has an acidic environment full of proteolytic enzymes. Proteins are often denatured or destroyed in the stomach, rendering them useless. The small amount of protein that makes it through the stomach intact then has to somehow be transported across the lining of the intestines to get into the bloodstream. In a healthy person, the transport is greatly reduced by layers of mucus and no significant mechanism of transport across the cell layers that line the intestines. These barriers lead to low concentrations of active protein in the bloodstream and this low bioavailability is also concerned with chemotherapeutic agents and other compounds administered across the intestinal epithelium.

In our laboratory, we have developed copolymers capable of protecting a protein while it is in transit through the stomach and then aiding in increasing the protein's transport across the cellular barrier in the upper small intestine. We have used insulin and calcitonin as model proteins. Insulin is a key protein in the treatment of diabetes mellitus and represents a protein used for a disease that affects a large number of people all over the world. Diabetes affects over 16 million people in the US alone. Calcitonin is used for the treatment of osteoporosis, a disease which affects over 10 million people in the US. In addition to this group, over half of the US population over the age of 50 has low bone mass, thereby elevating their risk of developing osteoporosis. In addition to these two proteins, we have also worked with bleomycin as a chemotherapeutic agent promising for oral administration. Cancer is the second leading cause of death in the US and one in three Americans will develop cancer during their lifetimes. There is a global demand for these treatments and successful development of oral administration methods for these agents would have a profound impact on the treatment of and the quality of life for the millions of patients suffering from these diseases.

2. Physiology of the gastrointestinal tract

Systems used for the delivery of therapeutic agents via the oral route must be designed conscious of the physiology of the gastrointestinal tract. The anatomy and physiology of the route of administration will dictate many of the requirements for the system.

For example, the device must be able to withstand the saliva, as saliva contains digestive enzymes and other reagents for breaking down whatever is placed in the mouth. The stomach, the main digestive organ of the body, contains many digestive enzymes and a very low pH. The pH of the stomach has been measured from 1.4 to 2.1 [3]. This harsh environment causes the destruction and denaturation of proteins delivered without protection. The pH of the stomach changes when food is present increasing to nearly 4.0 [3].

Once through the harsh conditions of the stomach a device reaches the small intestine which is divided into three regions. The first region, closest to the stomach, is the duode-

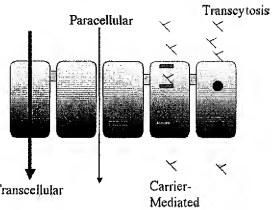

Fig. 1. Four mechanisms of transport across a cell monolayer.

num, followed by the jejunum and ileum. Fewer nutrients are taken into the blood stream the further down the small intestine they move. The duodenum, about 10 in. in length, composes the first 5% and the jejunum the following 40% of the length of the small intestine. The entire length of the small intestine is 5 m and the residence time within the organ typically ranges from 2 to 4 h.

The linings of the small intestine are composed of the serous, muscular, areolar, and mucous layers. Only the mucous and areolar layers are the important layers with respect to drug delivery. Transport of nutrient into the body occurs through the mucosal layer and into the areolar layer where the nutrients are taken into the blood stream. In the mucosal layer, there are cell layers that stick out of it and into the open area of the duodenum. These cell layers are arranged in villi and are where the majority of nutrients are absorbed into the body.

The transport of nutrients across the cell layer and into the blood stream can occur via four different transport mechanisms from one side of the cellular barrier to the other [4,5]. The first mechanism (Fig. 1) is transcellular and is primarily used by small molecules. The molecule diffuses from one side of the barrier, through the cell, and to the other side. Often these are molecules such as oxygen and carbon dioxide. The molecules are either too small or neutral in charge so they pass through the layer uninhibited.

The second mechanism of transport across cellular barriers is transcytosis. Molecules approach the cellular barrier and interact with the cell membrane. The membrane forms a pocket of lipid bilayer around the material, called vesicle. The vesicle detaches from the cell membrane and passes inside the cell. There are two fates for the contents of the vesicle, either it will move to the other side of the cell and be released through the other side of the cellular barrier, or it will be digested inside the cell.

The third mechanism is carrier-mediated transport. The molecule approaches the cell membrane and interacts with key groups on the surface of the lipid bilayer. The molecule reversibly binds to complexes in the bilayer, and the bound molecule-complex crosses the lipid bilayer to the inside of

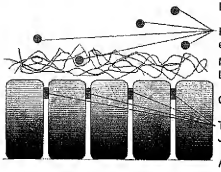

Fig. 2. Environment of the upper small intestine.

the cell. The molecule-complex disassociates and the molecule moves to the other side of the cell where a similar process occurs placing the molecule outside of the cell and across the cellular barrier.

The fourth and possibly most relevant mechanism of transport to this research is paracellular transport. Paracellular transport is when the molecules move through the layer by passing between adjacent cells. This movement is governed by the available space and environment between the cells. Increasing the area available between the cells allows the molecules to move more easily across the layer. Paracellular transport is the primary route used by hydrophilic and charged molecules.

The cell layer lining the small intestine is composed of epithelial cells, with a few endocrine cells interspersed throughout the layer. Fig. 2 is the cell layer in contact with the muscosal layer and lumen. It also shows the junctional complex, the junction between adjacent cells, possessed by the cell layer. Without it, transport of materials would freely flow between the cells from the lumen to the basal lamina or blood stream side. The junctional complex is divided into three regions: the abluminal component is the macula adherens or spot desmosome, the intermediate region known as the zonula adherens or intermediate junction, and the most lumen region, the zonula occludens also known as the occluding or tight junctions.

Tight junctions are important for drug transport of large molecules that do not have transporters or receptors on the epithelial cell's surface. They consist of a zone 100–600 nm in depth in which the lateral membranes of adjacent epithelial cells are closely apposed [6]. These fusion sites are the barriers to macromolecular flow across the epithelium. Tight junctions contain a series of fusion sites creating several barriers to flow, not just a single one. These tight junctions are the leakiest in the upper region of the small intestine, the duodenum, and become progressively tighter as passing towards the colon. This makes the duodenum an attractive site for release and potential absorption of a therapeutic agent.

3. Complexation hydrogels in oral delivery of therapeutic agents

Two major problems exist in developing oral delivery systems for therapeutic agents, specifically proteins and peptides [7–9]. The first problem is the inactivation of sensitive peptides by digestive enzymes in the gastrointestinal (GI) system, mainly in the stomach. This can be overcome by designing carriers which would protect the drugs from the harsh environments of the stomach before releasing the drug into more favorable regions of the GI tract, specifically the lower regions of the intestine (Fig. 3). Additionally, protease inhibitors could be used to retard the action of enzymes which could degrade peptides and proteins present in the GI system. The other problem is the slow transport of large macromolecules across the lining of the intestine into the blood stream.

We have developed hydrogen bonding-induced, complexation copolymer networks of poly(methacrylic acid) grafted with poly(ethylene glycol) (P(MAA-g-EG)) as oral delivery systems for a variety of therapeutic agents. The polymer complexes are prepared by free radical solution polymerization [10] or dispersion polymerization [11] of methacrylic acid and methoxy-terminated poly(ethylene glycol) monomethacrylate with tetra(ethylene glycol) dimethacrylate added to provide crosslinks in the network structure.

These materials exhibit pH-dependent swelling behavior due to the formation/dissociation of interpolymer complexes [12,13]. In acidic media, interpolymer complexes form due to hydrogen bonding between the graft PEG chains and the carboxylic acid protons. These complexes serve as temporary, physical crosslinks and cause the gels to exist in a collapsed conformation. The pK_a of PMAA is about 4.8, thus

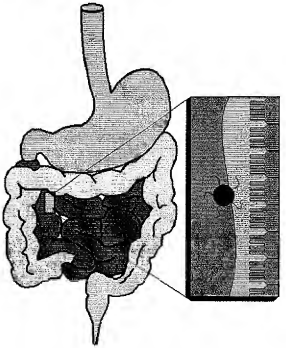

Fig. 3. The target site for delivery in the gastrointestinal tract is the upper small intestine. The inset box depicts release of a protein or chemotherapeutic agent into the intestinal lumen at the edge of the mucosal layer covering the intestinal epithelial cells.

at neutral pH the MAA groups in the network are almost entirely deprotonated. The hydrogen bonds present at low pH dissociate at neutral and high pH resulting in swelling of the network structure. The equilibrium swelling characteristics of the polymer network depend on the composition of the polymer. Lowman and Peppas [13] have studied the effects of copolymer composition and the pH of the surrounding medium on the network structure, as well as the drug release characteristics. Hydrogels comprising MAA and EG in equimolar amounts exhibit the greatest change in the mesh size or the correlation length of the network due to the pH shift. With the increase in the amount of MAA in the network, the average mesh size in the acidic media increases owing to the absence of physical crosslinks arising from MAA–EG interactions. Depending on the pH of the surrounding medium, the average mesh size in a network with a MAA/EG ratio of 1:1 changes by a factor of 3 between the collapsed and swollen states which corresponds to a 10-fold change in the effective area for diffusion of the drug. This results in a change in the diffusion coefficient of the drug by two orders of magnitude [14]. Thus these hydrogels are ideal for the oral delivery of therapeutic agents due to the large change in network structure over a small pH range. In the acidic environment of the stomach, the drugs would be trapped in the collapsed gel and protected from degradation by digestive enzymes in the stomach. However, in the neutral or basic environments in the intestine where the drugs could potentially be absorbed, they would readily be released. In addition, the macromolecular polymer structure and the polymer composition can be altered to achieve diffusion-controlled delivery of a wide range of compounds with therapeutic properties.

These complexation hydrogels can also inhibit the activity of Ca^{2+} dependent proteolytic enzymes [10]. Unpublished studies performed in our laboratories have also demonstrated the ability of these complexation hydrogels to protect the entrapped insulin in gastric fluid. Further, insulin released from the polymer formulations in intestinal fluid is significantly protected from the proteolytic attack. These polymer networks also exhibit mucoadhesive characteristics due to the PEG chains which interact with the mucus layer lining the epithelial cells. This mucoadhesive behavior is advantageous since it results in release of the drug at the site of absorption and also increases the residence time of the drug in the small intestine.

These characteristics make these copolymers ideal carriers for oral delivery of proteins and peptides. We have successfully demonstrated application of this system in oral delivery of insulin in diabetic rat models. Insulin can be incorporated into the polymer microparticles by partitioning from a concentrated solution followed by acidification to collapse the microparticles. Upon exposure to acidic environment most of the entrapped insulin remains trapped inside and less than 10% of insulin is released from the microparticles. But when the pH of the surrounding medium rises to the physiological pH in the small intestine, insulin trapped inside the network is released rapidly [15]. In addition to exhibiting a pH responsive behavior [16–18], these polymer matrices also serve to stabilize the entrapped insulin [17]. The PEG chains present in the network serve to maintain the biological activity of the insulin by stabilizing the drug and preventing binding to the ionizable MAA backbone [17,18]. The effectiveness of this system in delivering insulin was evident from the observed hypoglycemic effect following oral administration in the diabetic rat studies [19]. The blood glucose levels in diabetic rats were lowered by up to 40% for greater than 8 h. Transport studies using Caco-2 cells, a widely used in vitro model for intestinal absorption of drugs [20], have also demonstrated the efficacy of the polymeric system [21]. The particles induced Ca^{2+} concentration-dependent lowering on the transepithelial electrical resistance (TER) due to the reversible opening of the tight junctions [11,21], resulting in an increase in the permeability coefficient of insulin across the cell monolayer. Whether a similar permeation enhancing effect is responsible for the improved oral bioavailability of insulin in animal studies is not clear.

We have also examined the possibility of using the above mentioned polymer formulations for oral administration of the polypeptide hormone salmon calcitonin (sCT). Calcitonin is a 32-amino acid polypeptide hormone, which is in involved in the calcium metabolism in human beings and other vertebrates. This polypeptide hormone is used as a therapeutic agent for bone diseases such as Paget's disease, hypercalcemia, and osteoporosis. Like other proteins it is delivered mainly by injection, leading to a limited use in the treatment of osteoporosis.

Microparticles of P(MAA-g-EG) hydrogels loaded with sCT in a manner similar to that for insulin also exhibit pH-responsive release. Transport studies in Caco-2 cells have demonstrated that the polymer microparticles cause a significant increase in the permeability of sCT across the cell monolayer. The permeability coefficients of sCT in the presence or in the absence of the microparticles are shown in Table 1. It was observed that the hydrogels caused an increase in the paracellular permeability of sCT across the monolayer [22].

4. Other approaches in oral delivery of proteins

Approaches for improving the oral bioavailability of therapeutic proteins and peptides by transiently modulating

Table 1
Permeability coefficients for salmon calcitonin through Caco-2 cell monolayers at 37 and 5 °C for apical-to-basolateral (AB) and the basolateral-to-apical (BA) directions using P(MAA-g-EG) microspheres of the 4:1 ratio

Formulation (°C)	$P_{app} \pm 95\%$ CI (cm/s) $\times 10^8$
Control AB 37	10.98 ± 1.31
Control AB 5	3.02 ± 1.31
Control BA 37	11.12 ± 1.60
Hydrogel AB 37	23.52 ± 3.10
Hydrogel AB 5	9.02 ± 1.20
Hydrogel BA 37	18.35 ± 6.18

the tight junctions between the cells suffer from the possibility of side effects such as systemic toxicity and damage to the epithelium. The potentially invasive nature of this approach combined with the lack of precise control over the tight junction permeability limits its clinical applicability. We highlight here some of the promising alternatives to this approach that are currently under investigation for development of oral delivery formulations for proteins and peptides.

4.1. Receptor-mediated endocytosis for improved oral delivery

Receptor-mediated endocytosis of proteins and peptides is being investigated as a specific, non-invasive alternative to facilitate intestinal absorption of drugs [23,24]. Receptor-mediated endocytosis is a pathway for selectively and efficiently taking specific macromolecules required for various cell processes from the extracellular milieu. During this process, the binding of receptor to a ligand triggers intracellular signaling pathways which lead to endocytosis of the receptor–ligand complex in membrane-derived vesicles. After endocytosis, the receptor–ligand complex encounters the acidic pH in the early endosomal compartments [25], which may lead to uncoupling of the ligand–receptor complex due to changes in the conformation of receptor proteins followed by recycling of the receptor to the cell membrane or degradation of the receptor in lysosomes. Those endocytosed ligands that dissociate from their receptors in the early endosomes are usually degraded in the lysosomes. Some ligands, however, remain bound to the receptors and hence share the fate of their bound receptors. In polarized epithelial and endothelial cells, the process is called transcytosis and is of particular interest from a drug delivery standpoint because the complex is transported to a different domain of the membrane. By coupling proteins and peptides to ligands that can recognize specific receptors on the epithelial cell monolayers, transcellular delivery of these macromolecular biopharmaceuticals may be achieved. Since only those molecules that are conjugated to the ligands are transcytosed, this process eliminates the potential side effects associated to the unspecific transport by the paracellular pathway.

4.2. Lectin-mediated drug delivery

Lectins are a class of structurally diverse proteins of non-immune origin that are characterized by their ability to specifically bind carbohydrates [26]. They are primarily found in seeds, but are also present in animals, plants and microorganisms. Lectins have been studied widely for oral delivery applications because of their relatively good resistance to the acidic pH and enzymatic degradation and presence of binding sites in the gastrointestinal tract [27,28]. While some lectins such as Phascolus vulgaris agglutinin (PHA) from raw kidney beans have been known to cause dietary toxicity [29,30] and are unsuitable for drug delivery applications, a variety of other lectins such as wheat germ agglutinin (WGA) from *triticum vulgare* [29] and tomato lectin (TL) from *lycopersicum* bumin [31] are natural components of our diet and bind to the intestinal mucosa without any obvious toxic side effects.

WGA is a dimeric protein with two subunits containing two or four carbohydrate-combining sites. It specifically binds *N*-acetylglucosamine and sialic acid residues. Wheat germ contains approximately 300 mg WGA/kg and hence the peroral toxicity that can result from long term oral administration of WGA may be negligible. Further the lectin can resist degradation due to proteolytic attack upon exposure to the gastrointestinal enzymes [27]. These characteristics along with the abundance of *N*-acetylglucosamine-containing oligosaccharides in the glycocalyx of the intestinal mucosa make WGA a promising candidate for enhancing oral bioavailability of drug candidates.

Binding studies of WGA on Caco-2 cells, mucus-producing HT-29 cells and HT-8 cells has revealed that WGA exhibits low non-specific protein binding to the cell surfaces and high affinity for oligosaccharides present on the cell surfaces [32]. Gabor et al. [33] examined whether WGA can improve the binding and uptake of fluorescently labeled bovine serum albumin (F-BSA), used as a model protein, by the Caco-2 cells. They observed that WGA in the conjugated form was able to bind specifically to the oligosaccharides on the cell surface and the conjugate was taken up by the cells through an energy dependent mechanism. This is an important result since it demonstrates the potential of lectin-mediated delivery in improving the transcellular transport of proteins across the epithelium. However, there are concerns about the cellular fate of the absorbed conjugate. The conjugate accumulates within a lysosomal compartment where it is degraded. The degraded products diffuse into the cytosol and finally appear in the extracellular medium. Thus even though lectins can resist degradation due to proteolytic attack, the conjugated protein or drug is still susceptible to degradation by proteolytic attack in the lysosomes. In addition the stability of such conjugates in the gastrointestinal environment is an unaddressed issue.

One important consideration here is the size of the protein under consideration that is conjugated to the lectin. WGA is a 147 kDa protein whereas BSA is a 68 kDa protein. Since the two proteins have comparable sizes, the lectin may not be able to protect bound albumin by creating a steric hindrance to the proteolytic attack. But if a smaller protein such as insulin (5.8 kDa) is conjugated to WGA, the lectin might shield the bound protein from proteolytic attack and hence improve its stability during transit in the GI tract and through the cellular barrier. Nevertheless, results from the binding and uptake studies of BSA-WGA conjugate in cell culture models are promising and further studies with other proteins can answer many important questions in the approach.

4.3. Transferrin-mediated drug delivery

Transferrin (Tf) is a single-chain glycoprotein with molecular weight of approximately 80 kDa. Serum transferrin is

involved in transport of iron from the sites of intake to cells and tissues [34]. Transferrin receptors (TfRs) have been widely explored for receptor-mediated delivery of anticancer agents and in enhancing the transport of drugs across the blood brain barrier and more recently, across the epithelial cells of the intestine [35]. TfR is a homodimer expressed by many cell types including the human intestinal cells. TfR binds iron-bound transferrin (holo-transferrin) on the cell surface. This binding is sensed by the cells which results in uptake of the Tf-TfR conjugate. Triggered by the change in the endosomal pH, the complexed transferrin then looses its bound iron and the complex is recycled to the cell surface where iron-free transferrin (apo-transferrin) is released. In polarized cells, transferrin can be transcytosed from the apical to basolateral membrane. This mechanism is exploited in TfR-mediated drug delivery across the blood brain barrier as well as the epithelial barrier of the small intestine.

Transferrin receptors are present in high density in human GI epithelium, and transferrin can resist tryptic and chymotryptic degradation. Shen and co-workers [36] were the first ones to realize the potential of TfR-mediated transport in improving oral delivery of therapeutic agents. They conjugated transferrin to insulin via disulfide linkages and demonstrated that the conjugation of insulin to transferrin resulted in a 5- to 15-fold increase in insulin's transport across Caco-2 cell monolayer. As pointed out earlier, since this enhancement is specific for the conjugated protein, and takes place without causing the tight junctions to open even momentarily, this approach is most desirable in terms of toxicity and damage to the epithelium.

Following successful in vitro studies, Shen and co-workers [37,38] demonstrated that an insulin-transferrin (In-Tf) conjugate, when administered orally to diabetic rats, caused a slow but prolonged hypoglycemic effect. In-Tf conjugate after oral administration as well as after subcutaneous injection caused a delayed but prolonged glucose-reducing activity as compared to native insulin after subcutaneous injection. Subcutaneously-administered In-Tf conjugate maintained low blood glucose levels at least four times longer than unconjugated insulin. While this might be beneficial in terms of the frequency of dosage, the reasons behind this behavior need to be investigated. One promising explanation suggested by the authors that is consistent with the observations is that the conjugation of insulin to transferrin may result in the longer plasma half-life of insulin.

The authors have also presented data suggesting that the disulfide linkage between the two proteins can be reduced in the liver following its absorption into the portal vein. The approach thus makes use of transferrin for overcoming the transport barrier of the small intestine after which free insulin can be released to perform its physiological function.

The observation that the intact conjugate is able to reach the systemic circulation intact, suggests that the insulin conjugated to transferrin is able to overcome proteolytic attack during transit in the GI tract and across the epithelial cells. In vitro studies with simulated gastric and intestinal fluids can shed light on the conjugate's ability to shield insulin from proteolytic attack.

5. Oral chemotherapy

Oral delivery of chemotherapeutic agents is only available for a small number of drugs but has shown some promising results when compared to the conventional intravenous administration. Some of the agents that have been used in oral treatment of cancer include hormones, (tamoxifen), taxanes, (paclitaxel), epipodophyllotoxins, (etoposide), antimetabolites, [5-fluorouracil (5-FU)], and camptothecin derivatives, (topotecan). Recent clinical studies, using these agents comparing oral to intravenous administration found that not only was the toxicity lower with oral dosage forms but clinical outcome was comparable, and in some cases improved, with oral administration [39–43].

The advantages of oral chemotherapy go beyond survival time and toxicity. Lower cost, increased patient compliance, flexibility of dosing schedule and an overall improvement in quality of life are additional benefits of oral chemotherapy. A recent study concerning patient preference showed that the large majority, approximately 90%, of cancer patients preferred oral administration to intravenous administration [44]. Even without an improvement in efficacy or toxicity, oral chemotherapy would be a significant advance in the field of oncology.

5.1. Clinical evaluation of oral dosage forms: experiences with topotecan and vinorelbine

One chemotherapeutic agent that has undergone numerous studies in oral dosage forms is topotecan, a camptothecin analogue. Camptothecins are an attractive candidate for oral therapy because their durations of treatment are extensive and the highest drug efficacy is shown when tumors are exposed to the drugs at low levels for long periods of time. This plasma profile is best obtained by intravenous infusion or daily dosage. Due to the low quality of life and high cost of prolonged infusion administration, oral dosage is a fitting delivery strategy for this class of chemotherapeutics. The potential for oral dosages forms is further underscored by the conclusions of a recent review of topotecan treatment of relapsed ovarian cancer [45]. Herzog discusses the results of phase II and III trials with topotecan when administered by the parenteral route and concludes that topotecan has similar outcomes with paclitaxel and pegylated liposomal doxorubicin but that an alternative dosing schedule could lead to further improvement.

The motivation for changes in the dosing schedule is due to the toxicity that can result from infusion of topotecan with myelosuppression being the most severe toxic response. Because older patients are at a higher risk to develop a toxic response to topotecan, a recent phase II study sought to evaluate the efficacy of a reduced dose of the drug for treat-

ment of myelodysplastic syndrome [46]. The standard dosing schedule of 2 mg/m^2 i.v. infusion over 5 days was reduced to 1.5 mg/m^2 i.v. infusion over 3 days. The hope was to provide a similar response rate with reduced toxicity but only one of the 15 patients enrolled exhibited a complete remission. This represented a marked reduction from the expected response with the standard therapy. The conclusion of this report was that the dosing schedule used had no clinically significant activity but the need for a better system remains. Given the success of topotecan in the treatment of a variety of malignancies and the potential for improvement with dosing schedules that take advantage of its high efficacy with prolonged exposure to the tumor, a successful oral dosage form for topotecan would be a welcome addition to the options presented to clinicians for the treatment of these malignancies.

While the issue of topotecan delivery is still an area of research, the need for an oral dosage form was recognized years ago and some studies have already been completed. A preclinical study was completed to directly compare the efficacy of topotecan when administered orally or intravenously to athymic nude mice with human xenografted tumors [47]. The results of this study showed the oral group had clinical outcomes on par with the i.v. group for three tumor types and improved outcome compared to i.v. in the other four tumor types studied. While the bioavailability was only 23.5%, the oral group received a higher cumulative dose and the prolonged exposure was thought to lead to the improved results. The oral group had higher incidences of weight loss than the i.v. group but instances of lethal toxicity was reduced from 9% to 2% in the oral group indicating that the increased dose over an extended period did not increase the risk of this therapy. This study showed a clear therapeutic advantage for the daily oral topotecan. The additional benefits of higher patient compliance and quality of life as well as reduced cost with oral therapy only add to the therapeutic benefits.

Gore et al. [39] analyzed the effect of topotecan when given orally as opposed to the intravenous regimen for patients with ovarian cancer. Their results showed that the grade IV toxicity was reduced from 51% to 15% in the oral group, and the median overall survival factor decreased to 51 from 58 weeks. Further analysis showed that the reduced amount of oral topotecan in the bloodstream compared to the intravenous injection kept the concentration of drug at lower levels possibly leading to the decreased toxicity as well as the slightly worse survival time. Future studies plan to increase the amount of topotecan given orally to see if the survival time can be increased while maintaining comparable or decreased toxicity relative to intravenous administration. The delay in reaching the bloodstream due to the need for absorption when using an oral dosage may decrease the spike in plasma concentration of drug when given as an injection with positive results for both efficacy and toxicity.

Numerous other chemotherapeutic agents have undergone clinical evaluation to assess the efficacy and safety of oral administration. In addition to a number of studies that have shown very promising results such as those seen with topotecan, there are others where other agents or new approaches will be necessary. A multi institute study on oral administration of vinorelbine evaluated the oral dosage form clinically as well as gathering data from the patients on their preference for oral therapy [48]. Vinorelbine has been shown in a pair of trials to be an effective treatment for elderly patients with metastatic non-small cell lung cancer when given by the parenteral route [49,50]. An oral dosage form of vinorelbine was approved in 1994 and Kanard and colleagues found minimal efficacy in a phase II study of this dosage form in elderly patients with metastatic non-small cell lung cancer. While the i.v. treatment exhibited response rates of approximately 20%, the response rate with the oral dosage form was only 3.4%. The treatment was well tolerated by the patients but the lack of activity against the tumor prevents it from being a viable alternative to the intravenous route of administration. While the results of the clinical evaluation of the dosage form were disappointing, the patient preference results underscore the previous study mentioned above that showed the demand for oral chemotherapy options [44]. Briefly, when patients were asked initially and 1 month into the therapy whether they prefer chemotherapy pills to intravenous chemotherapy, 96 and then 97% replied that they did prefer the pills. While the authors acknowledge potential bias towards preferring the oral option in the subject group, the results are a fairly convincing endorsement of the oral route. Despite the poor efficacy of this trial, the demand for oral dosage forms will continue to motivate future studies.

5.2. Factors affecting bioavailability of chemotherapeutic agents

Many of the chemotherapeutic agents given orally do not have as high a bioavailability as topotecan and methods to increase their absorption are under investigation. In fact, the bioavailability of most chemotherapeutics is very low and also variable when administered orally. These difficulties create higher cost as well as risk of an inappropriate dose due to unpredictable uptake. Some of the mechanisms leading to these problems and potential solutions will be discussed here. For a more in depth review of this subject, we refer you to the review of Schellens et al. [51] on the differences in oral bioavailability of chemotherapeutics when moving from mouse models to clinical trials.

There are a number of mechanisms by which the drugs can be inactivated or prevented from reaching the bloodstream. The possibility of agents passing from the intestinal lumen through the epithelial cell layer and into the bloodstream was discussed earlier but this is not a simple process. First, the intestine has numerous enzymes present that can inactivate both protein agents as well as many other anticancer drugs. The epithelial cells that line the intestine have a number of transporter proteins on their surface that can send drugs that were able to enter the cell back out into the lumen as opposed to passing through the cell towards the bloodstream. The

specific mechanisms by which these processes occur are under investigation to devise strategies to overcome them and increase the bioavailability of orally administered compounds.

One of the anticancer agents whose bioavailability is affected by the presence of enzymes is 5-FU. Dihydropyrimidine dehydrogenase (DPD) is involved in the metabolism of numerous pyrimidines and can make oral administration of 5-FU ineffective. A recent study by Baker [52] found that the use of the DPD inhibitor eniluracil increased the bioavailability of oral 5-FU to levels approaching 100%. Lowering the activity of enzymes is also of concern when using proteins or peptide drugs as many enzymes in the small intestine are designed to break proteins down into mono-, di- or tripeptides for subsequent absorption by the epithelial cells and transport into the bloodstream. This would obviously eliminate any potential therapeutic potential for the protein and lead to bioavailability values that are unacceptably low.

Another potential cause for low bioavailability for orally administered chemotherapeutic agents is drug efflux from the epithelial cells back into the lumen by intestinal drug transporters. Two of the major drug transporter families are the *P*-glycoproteins and multidrug resistance-associated proteins (MRP1, 2, and 3). *P*-glycoproteins are primarily expressed on the apical membrane of epithelial cells and the lumen side of the intestine [53]. MRP1, 2 and 3 are all expressed in the intestine with MRP2 expressed highly in the duodenum [54]. These transporters are used primarily to detoxify the cells but can bind anticancer drugs and return them to the intestinal lumen, thereby preventing their entry into the circulation. For particular drugs that are known to be substrates for transporters, studies are underway to investigate the effect of inhibiting the transporters on bioavailability. The bioavailability of the anti-mitotic agent paclitaxel, given orally in combination with the *P*-glycoprotein inhibitor cyclosporin A, can be modulated by the amount of cyclosporin A [55]. Related work by Hoffmeyer et al. [56] showed that mutations in *P*-glycoprotein that reduced its expression in the duodenum led to increased plasma concentrations of *P*-glycoprotein substrate drug, digoxin, when given by oral administration.

Some other studies utilizing oral administration of chemotherapeutic agents have hinted at the potential that lies in the field but also illustrate the need for more trials and better delivery systems. More work and better systems are required because there are a number of obstacles to oral delivery that must be overcome for the system to be effective. The most obvious when comparing this method of administration to intravenous injection is getting the therapeutic agent from the gastrointestinal tract into the bloodstream. For intravenous injections the bioavailability is high because it is administered directly to the bloodstream. For oral administration, the compound must be released from its carrier and pass from the gastrointestinal tract to the bloodstream without being inactivated. How low the bioavailability is depends on the susceptibility of the agent to the conditions of the gastrointestinal tract and its ability to pass out of the tract into the bloodstream. It is safe to say the bioavailability will be lower than intravenous injection but the magnitude of this decrease varies between compounds and on the method of oral administration.

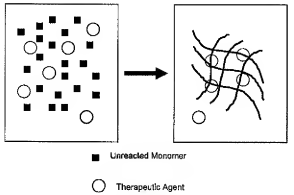

Fig. 4. As the polymerization takes place, the free therapeutic agent becomes trapped within the hydrogel network with its diffusion controlled by the state of the network (collapsed vs. swollen).

When dealing with chemotherapeutic agents, another concern regarding the agent in the agent in the gastrointestinal tract is the potential toxicity of these agents. Not only does the agent have to be protected from the low pH of the stomach and degradative enzymes present, but also the lining of the gastrointestinal tract must be protected from the potentially cytotoxic agent. If the chemotherapeutic agent is contained within its carrier until it reaches the site of release and, hopefully, absorption, then the toxicity of the agent to the tissue at this site must be investigated. If the agent produces unacceptable levels of toxicity at this site, the site of delivery must be changed or this agent will be deemed unsuitable for oral administration.

5.3. Use of hydrogels as oral delivery systems for chemotherapeutics

Hydrogels can be used for delivery of a variety of therapeutic agents. The previous discussion has focused on the use of hydrophilic polymer carriers for oral delivery of proteins such as insulin [14]. We also showed that a carrier that is capable of increasing the bioavailability of chemotherapeutics could aid current efforts to develop oral dosage forms for cancer patients. The loading of proteins into the hydrogels was done by imbibition, where the polymer is swollen in a solution containing the protein and collapsed at low pH to trap the protein inside. An alternative method is in situ polymerization loading. In this method, the agent is diluted into the initial monomer mixture prior to polymerization. As the polymerization reaction takes place and the polymer forms, the diluted agent is trapped inside the polymer network that forms around it. An illustration of this process is shown in Fig. 4. The in situ method can only be used with agents that will not lose activity during the conditions of the polymeriza-

tion reaction, which eliminates most proteins when a UV-initiated polymerization is used due to UV-denaturation. This method has been used for loading of chemotherapeutic agents into the hydrogels with most of the research focused on delivery of bleomycin.

There are a number of issues associated with oral delivery systems including protection of the agent from the gastrointestinal tract, cell–polymer interactions and transport of the drug from the gastrointestinal tract into the bloodstream. This transport is a critical step and a site favorable for absorption along the gastrointestinal tract must be chosen. Recent work in our laboratory for delivery of bleomycin focuses on the duodenum as a favorable site for absorption from the GI tract. P(MAA-g-EG) hydrogel carriers were synthesized by a free radical polymerization. Bleomycin was loaded into these carriers by performing an in situ polymerization with an efficiency of 76% (±9% $n = 3$). Analysis of the final product shows 1 wt.% bleomycin is incorporated into the hydrogel in its dry state. Release studies were carried out in conditions to model the environment of the stomach and small intestine. Results showed that bleomycin is preferentially released at a higher pH due to the increased mesh size of the swollen hydrogel carrier. Cell–polymer interaction and transport studies are still underway.

6. Conclusions

There are numerous strategies currently under investigation to effectively deliver a range of therapeutic agents from the gastrointestinal tract into the bloodstream. These efforts are fueled by the preference of patients for oral dosage forms as well as the improved efficacy and toxicity for certain classes of agents. Successful endeavors in this area will be the result of combining knowledge from a variety of disciplines including, but not limited to, materials science, molecular biochemistry and pharmacology. Our research focuses on the use of environmentally-sensitive hydrogels to serve as carriers for a range of proteins and chemotherapeutics but many other possibilities exist and some of these have been discussed above. Further development of these systems will hopefully expand the scope of agents capable of being administered orally while also improving the efficiency of delivery for some agents currently given by the oral route.

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